

Policy & Procedure (P& P)

Policy Title :

Cross-Matching Procedure

Department	Index No.	Scope
Laboratory & Blood Bank	LAB-125	All Blood Bank Staff
Issue Date	Revision NO	Effective Date
01/06/1433	3	24/08/1440
Review Due Date	Related Standard NO.	Page Number#
24/08/1442	CBAHI (LB.65)	8

01. Policy:

The cross-match is a test performed between the donor's red cells and recipient's serum prior to transfusion. It should exclude any ABO incompatibility or clinically significant antibodies in recipient serum that are directed against the donor's red cells. It is a job of great responsibility.

The permissible error is "0 %" This means that every work has to be done with great care according to standard.

Now we do, Rh subgroup & Kell phenotyping & trying to match between patient & donors blood type in this aspect, but up till now there is no clear memo from MOH about the use of alternatives in case unavailability of these antigens (Rh subgroup & Kell antigen).

02. Definition :

02.1. N/A

03. Purpose :

03.1. This policy aims to clarify and standardize the procedure for performing major cross matching to ensure issue of safe and compatible blood for transfusion.

04. Procedure :

We can do Cross-Matching procedure by the following methods

- A) Tube method.
- B) column technology & the Gel Micro typing system.
- C) Tango Optima (Fully automated machine)

A. A) Tube method.

1. Specimen Requirements

Fresh serum obtained from a fully clotted specimen should be used. Incompletely clotted blood samples may cause small fibrin clots that trap red cells into aggregates that resemble agglutinates. Samples with high levels of fibrinogen may induce rouleaux formation that could be mistaken for agglutination.

1.1 Appearance of Sample:

The appearance of sample may create difficulties in detecting antibody induced hemolysis. Whenever possible hemolyzed sample should be replaced with a new sample. Test results observed with lipemic samples can also be difficult to evaluate.

1.2 Age of Sample:

Blood samples intended for use in cross-matching should be collected no more than 3 days before the intended transfusion. This is to ensure that the sample used for testing reflects recipient's current immunologic status because any recent transfusion may stimulate production of unexpected antibodies.

1.3 Retaining and storing blood samples:

The recipient's blood specimen must be stored at refrigerator temperature for 7 days. They must be available for retyping of recipient blood and re-crossmatching should be done if a transfusion reaction occurs.

2. Materials:

- 2.1 Glass test tubes.
- 2.2 Pasteur pipettes.
- 2.3 Isotonic saline 0.85% NaCl.
- 2.4 Calibrated serologic centrifuge.
- 2.5 37° C water bath.
- 2.6 Timer.
- 2.7 Microscope and slides.

3. Reagents:

- 3.1. 22% bovine albumin.
- 3.2. Anti-human serum for the anti-globulin test.
- 3.3. Coombs control cells.
- 3.4. Anti-A
- 3.5. Anti-B
- 3.6. Anti-D

- 3.7. Anti-E.
- 3.8. Anti-e.
- 3.9. Anti-C.
- 3.10. Anti-c
- 3.11. Anti-K

4. Method:

- 4.1 As the blood bag is removed from the blood bank shelf, the plasma must be inspected for evidence of hemolysis.
- 4.2 The expiration date of the blood must be noted to avoid issuing out dated blood.
- 4.3 Donor's and recipient's blood must be screened for atypical antibodies in advance of transfusion.
- 4.4 Wash both donors & recipients red cells and Prepare 2-5% suspension of donor's red blood cells and 2-5% suspension of recipient's cells for ABO and Regroup & subgroup & Kell antigen phenotyping.
- 4.5 Add 2 drops of patient's serum and 1 drop of suspension of donor's cells to a labeled test tube.
- 4.6 Mix and centrifuge.
- 4.7 Examine for hemolysis & agglutination, then gently re-suspend the cell button and examine for agglutination macroscopically and microscopically record results.
- 4.8 Mix, add 2 drops of 22% bovine albumin and incubate at 37°C for 15-30 minutes.
- 4.9 Centrifuge and examine for hemolysis & agglutination. Gently re-suspend the cell button and examine for agglutination macroscopically and microscopically record results.
- 4.10 Wash the cells at least three times.
- 4.11 Add 2 drops of antihuman globulin reagent.
- 4.12 Mix and centrifuge.
- 4.13 Gently re-suspend the cell button and examine for agglutination both macroscopically and microscopically. Record Results.
- 4.14 Add 1 drop of comb's control cells to the negative tubes. Mix and centrifuge. A positive agglutination test should be obtained for a negative cross-match.

5. Reporting Results:

3.1 Interpretation

- 3.1.1 A compatible cross-match is recognized by the absence of agglutination at any phase of testing.
- 3.1.2 Agglutination at any phase of testing indicates a serological incompatibility and the

cause must be investigated.

3.2 Reporting Results

6. Limitations:

5.2 If the patient is found to have clinically significant antibodies, units issued for transfusion should be non-reactive for such antigens when tested with licensed reagents.

5.3 Red cell units selected for transfusion to a patient with a potentially clinically significant antibody should whenever possible be tested and found negative for the appropriate antigen. Even if the antibody is no longer detectable, the red cells of all subsequent transfusions to that patient should lack the antigen even though red cells possessing those antigens are presently compatible in vitro. This is to prevent a secondary immune response.

5.4 Clinically significant red cell antibodies may become undetectable in a recipient's serum over time. Between 30% and 35% of antibodies become undetectable within 1 year and nearly 50% become undetectable after 10 more years.

5.5 The least incompatible blood is given in case of autoimmune hemolytic anemia after written approval from the physician.

7. Quality Control:

Reagents are to be tested with appropriate positive and negative controls daily and the results recorded. See "Daily Quality Control" for procedure

B. Cross matching using column technology & the Gel Micro typing system

1. Principle:

A specific red cell solution is added to the gel contained in a special micro tube. The gel acts as a trap, the free red blood cells pellet in the bottom of the tube while agglutinates are trapped(fixed)in the top of the gel for hours.

2. Equipment/Materials:

- 2.1. ID Centrifuge.
- 2.2. ID Incubator 37 C
- 2.3. ID working table.
- 2.4. ID pipettor EP-3/FP-2/FP-3.
- 2.5. ID Dispenser.
- 2.6. ID Suspension tubes.
- 2.7. ID Disposable tips.

3. Reagents:

ID Diluent 2(modified LISS).

4. Micro typing cards:

LISS/Coombs (Profile: -6 Coombs tests).

5. Sample:

5. 1. Red cell concentrate/Whole blood from donor unit.
5. 2. Serum or plasma from recipient.

6. Test procedure:

- 6.1. Allow all reagents to reach room temperature before use.
- 6.2. Identify the ID-micro typing card with the patient name and number and each micro tube with the donor unit number. remove the aluminum foil.
- 6.3. Prepare a 0.8% suspension of donor unit red cells as follow:
 - 6.3.1. 10-micron red cell concentrate + 1 ml ID- diluents 2 or
 - 6.3.2. 20-micron red cell concentrate + 1 ml ID- diluents 2 mix well.
- 6.4. Add 50 U of each donor unit red cell suspension to the appropriate micro tube.
- 6.5. Add 25U of recipient serum or plasma to the micro tube.
- 6.6. Incubate the micro typing card for 15 minutes at 37c in the ID-Incubator.
- 6.7. Centrifuge the micro typing card for 10 minutes in the ID-centrifuge.
- 6.8. Interpret the result.

7. Interpretation:

- 7.1 1Positive reaction, grade 1-4, in the micro tube indicates an incompatibility between donor and recipient. further investigation using Antibody identification panel by the appropriate technique, will be required to confirm the presence of antibodies.
- 7.2 Negative reaction in the micro tube indicates that the donor and recipient are compatible.

8. Quality Control:

Reagents & microtubes are to be tested with appropriate positive and negative controls daily (ID-Internal Quality Control) and the results recorded. See "Daily Quality Control" for procedure.

C. Cross matching using TANGO optima System (Fully automated machine)

1. Reagents

- 1.1. **Solid screen™ II Strip** Plates with 12 strips per plate. The strips are coated with protein A.
- 1.2. **MLB 2** Modified LISS 2 (Low Ionic Strength Solution) is an antibody enhancement media specifically formulated for use with the Solid screen™ II
- 1.3. test system, The MLB 2 is used in the preparation of a1% cell suspension of sample red blood cells.
- 1.4. **Alsevers Solution** Used as a suspension medium for red blood cells.

1.5. **Anti-Human Globulin (Anti-IgG Solid screen™ II)** The Anti-Human Globulin is used to demonstrate the presence of antibodies bound to the donors red blood cell that do not cause direct agglutination.

2. Materials:

- 2.1. Glass test tubes.
- 2.2. X rack of the Tango Optima machine.
- 2.3. Calibrated serologic centrifuge.

3. Sample:

Patients serum & Donors sample is taken from donor's bag segment
Both patients & donors' samples must be well centrifuged.

4. Principle of the test

Solid screen™ II Strip plates are composed of test strips (12 strips with 8 wells each) that have been coated with Protein A. Protein A has a high binding affinity for the Fc region of immunoglobulins. The method used for the Cross matching test on the Solid screen™ II Strip is solid phase adherence. If red blood cell alloantibodies and/or autoantibodies are present in the sample to be tested they will bind to the donors red blood cells. Unbound antibody is removed by washing. After adding Anti-Human- Globulin specially formulated for the Solid screen™ II assay and centrifugation Anti-Human-Globulin forms a link between the protein A on the surface of the microwell and the donors red blood cells. Thereby a layer of red blood cells will form on the surface of the microwell (compatible). But if no red blood cell alloantibodies or autoantibodies are present in the sample, the cells will not get coated with antibodies and therefore will not form a cell layer on the surface of the microwell but sediment to the bottom of the well to form a cell button.

5. Test procedure:

- 5.1. Centrifuge Patients & donor blood sample & put them in (X) Rack.
- 5.2. Open the sample door & put the (X) Rack in its correct place.
- 5.3. Push the Rack button to select the desired rack on the screen.
- 5.4. Press manual entry button & write the ID number of patients & donors' sample.
- 5.5. Press accepted entry button
- 5.6. Press (Cm) button to select the test & close the sample door.
- 5.7. Press start button to start the test.
- 5.8. After the end of the test validate the results.
- 5.9. Print out the results.

6. Quality Control:

Control Set QC contain test erythrocytes with defined antigen pattern for

ABO, Rh(D), phenotyping (Rh(CE) and Kell) as well as isoagglutinin's for reverse typing,

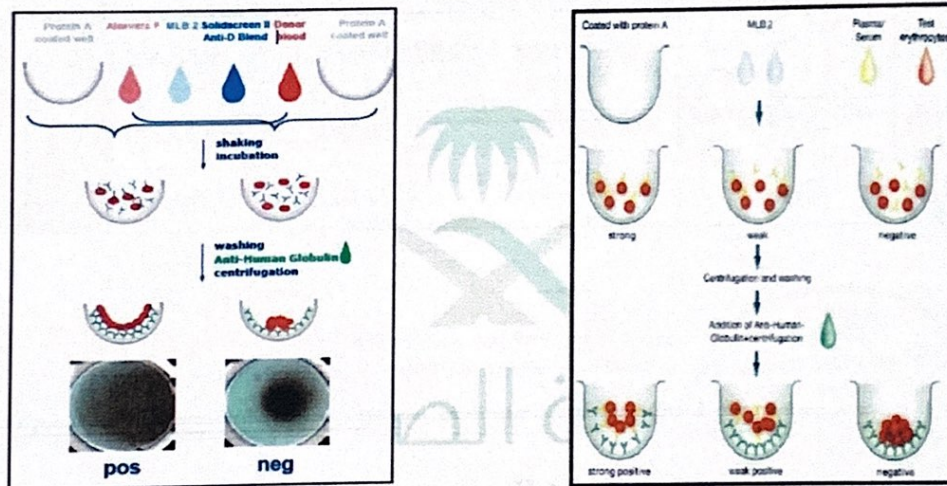
5.10. Group O Neg

5.11. Group AB Pos

5.12. Group A neg

5.13. Group O Pos

5.14. Solid screen 11-Control is used for Antibody screen QC.



05. Responsibilities :

05.1. All Blood Bank Staff of Al-Qunfudah General Hospital

06. Equipment & Forms

06.1. Cross-Matching Book

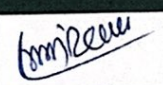
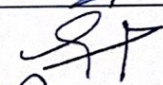
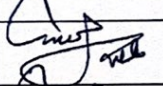
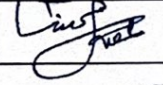
07. Attachment :

N/A

08. Reference

- 08.1. King Abdul Aziz Hospital.
- 08.2. Tango Optimo manual book
- 08.3. Manual for BB in arab countries.

Preparation, Reviewing & Approval Box

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